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Intermittent Hypoxia Elicits Prolonged Restoration of Motor Function in Human SCI

PRINCIPAL INVESTIGATOR:

Gillian Muir DVM, PhD

CONTRACTING ORGANIZATION:

University of Saskatchewan

Saskatoon, SK S7N 5A2

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14. ABSTRACT This research is part of a concurrent set of studies involving animals and human spinal cord-injured (SCI) subjects designed to test the effects of a novel therapy, termed acute intermittent hypoxia (AIH), on voluntary limb function following chronic SCI. The current research investigates the effect of AIH treatment in a rat model of cervical SCI. Over the 2 years of this study, we have determined that AIH, in combination with daily motor training in the form of a ladder-walking task, elicits sustained improvement in skilled limb use during ladder walking task in a rat model of SCI when compared to normoxia-treated, motor-trained control rats. In a separate experiment, spinal-injured rats treated with AIH without concomitant motor training did not show recovery on the ladder task. As well, it appears the motor training must be task-specific, in that rats receiving AIH in combination with treadmill training did not show functional recovery on the ladder-walking task. We also report that AIH does not facilitate recovery of grip strength or spontaneous forepaw use. These findings are important because they reveal that we can obtain consistent effects in an animal model for a promising SCI therapy. This therapy is also feasible, in that AIH has already been shown to augment motor function in persons with SCI.					
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INTRODUCTION

The long term goal of this research was to improve functional recovery in persons with cervical spinal cord injury. The specific purpose of our research was to assess the effectiveness of a novel therapy, termed acute intermittent hypoxia (AIH), to produce functional recovery in a rat model of cervical SCI. This therapy is based on the established finding that AIH (repeatedly breathing brief periods of low oxygen alternating with normal levels of oxygen) will strengthen synapses onto respiratory motoneurons by a mechanism known as long-term facilitation¹. Briefly, exposure to hypoxia is known to trigger oxygen-sensitive chemoreceptors which activate brainstem neurons. This results in increased protein synthesis in respiratory centres in the spinal cord, which in turn augments neural activity and improves respiratory function¹. In our preliminary data, we found that AIH also improves non-respiratory motor function in that forelimb function in spinal-injured rats and lower limb muscle activation in spinal-injured humans was improved with AIH exposure. Thus, over the 2 year scope of this research project, we proposed a concurrent set of studies involving animal and human experiments that provide a framework to quantify the effect of AIH on voluntary limb function following chronic spinal cord injury. The animal experiments quantifying functional recovery are the focus of the current report. Specifically, we proposed to (1) test the hypothesis that daily AIH (dAIH) elicits robust and prolonged improvement of voluntary limb function after chronic spinal injury (**Aim 1, Year 1**) and (2) to test the hypothesis that combining dAIH with motor training enhances and prolongs improvements in voluntary limb function after chronic spinal injury (**Aim 2, Year 2**). In rats with chronic cervical injuries, we planned to assess multiple indicators of limb function, including horizontal ladder performance, grip strength, and maximal treadmill speed, both before and after exposure to AIH. In the second year of this project we proposed to combine dAIH with daily motor training, with the rationale that together these therapies may elicit a greater degree of recovery than either alone. We expected that the knowledge gained from this translational study will assist in the development of future clinical trials with an end goal of improving motor function in spinal-injured humans.

BODY

As per our Statement of Work, our research efforts during Year 1 focused on **Specific Aim 1**, *to test the hypothesis that dAIH elicits robust and sustained improvement of voluntary limb function after chronic SCI*. Specifically, in **Task 1** we aimed to quantify the effect of dAIH on limb function in rats with chronic cervical injuries. We have completed all of the subtasks (Subtasks 1a through 1e) identified for Task 1, which include the behavioural conditioning, surgery, limb function assessments, AIH treatment and post-treatment assessments in 60 rats. In order to maintain feasibility and quality of work, we carry out our experiments using sub-groups of 12 rats at a time. In year one, we carried out 6 experiments (6 sub-groups of rats x 12 rats/gp = 72 rats); however, methodological complications were encountered early during these experiments and therefore not all of the data obtained could be used to address Aim 1 (as reviewed in the midterm report). These complications were resolved and we were able to obtain results in experiments using the ladder-walking task and grip strength measurements.

Research efforts during Year 2 focused on **Specific Aim 2**, *to test the hypothesis that combined dAIH and locomotor training enhances and prolongs improvements in voluntary limb function with chronic spinal injury*. Specifically, in **Task 5** we aimed to quantify the effects of dAIH combined with treadmill training on limb function in rats with chronic cervical injuries. In year 2, we carried out 6 experiments (6 sub-groups of rats x 12 rats/gp = 72 rats). Although we again encountered some methodological complications (outlined below), we have completed all of the subtasks (Subtasks 5a through 5d) identified for Task 5, which include behavioural conditioning, surgery, limb function assessments, AIH treatment and treadmill training, and post-treatment assessments in rats.

Methodological Complications:

Problem: Treadmill training caused a stress response in Lewis rats

One of our tasks for this study was to quantify the effects of dAIH, in combination with daily treadmill training, on limb function in rats with chronic cervical injuries (Task 5 under Specific Aim 2). We proposed to examine the effect of dAIH treatment combined with daily treadmill training to determine whether both treatments together could improve recovery of locomotion on the ladder walking task. We had also proposed to use maximum treadmill speed as an outcome/indicator of recovery. Two replicate experiments were performed combining daily AIH/control normoxia treatments with daily treadmill training/no treadmill training in a complete block design (n=4/group). Prior to surgery and treatments, rats were acclimated to the treadmill in order for them to become familiar and comfortable in the apparatus. This involved placing rats on the motionless treadmill for approximately 20 minutes per day for 3 days, followed by a few days of placement on the treadmill while it was moving at a slow speed and finally placement on the treadmill moving at the maximum speed intended for use during the training period. A food treat, vanilla icing, was placed at the end wall of the treadmill to entice rats to maintain their position on the treadmill, as we have used previously². While early acclimation trials were successful, rats increasingly showed signs of stress with subsequent trials, manifest as nasal and ocular porphyrin secretions, and piloerection. It is not clear why treadmill locomotion elicited a stress response in this group of rats. While we have previously used this method successfully with Long-Evans rats, it is possible that the Lewis rats used in the current experiments respond differently from other strains. We completed 2 replicate experiments where treadmill training was utilized in combination with treatment. Because of the unwanted effects of the treadmill training on Lewis rats, we chose not to use maximum treadmill speed as an indicator of recovery, as we had initially proposed, and instead, we have added a test of spontaneous forepaw use, the paw preference task, as described below.

Problem: Grip Strength Testing provides unreliable results in Lewis rats

Forepaw grip strength was proposed as one of our functional indicators of recovery, as this is a common test used in mice for toxicology testing, and is thought to mimic hand grip strength testing in human subjects. In rodents, this task was originally designed to test the grip strength of both paws. Positioning rats to test one paw at a time is difficult and uncomfortable for the rats, particularly those used to gentle and consistent handling as rats in our lab are. We found that these rats quickly became agitated and did not grip the bar consistently. We initially proposed using this task to assess grip strength after SCI in rats that had undergone AIH treatment alone (under task 1,

performed in year 1) and rats that had undergone AIH plus daily treadmill training (under task 2, performed in year 2). We present data on grip strength using both of these paradigms, as well as an additional paradigm (Figures 2, 4 and 7), although the task does not reliably assess unilateral grip strength in Lewis rats.

Solution: Addition of Paw Preference as a non-stressful, spontaneous paw use task

Due to problems with the treadmill training and the grip strength tasks, we substituted a different motor task, the paw preference task³. This well described task assesses the spontaneous use of the forelimbs during exploration of a novel environment. Digital video recordings are made while rats contact the walls of a cylindrical enclosure with their paws. The recordings are analyzed to determine the number of wall contacts made with the left and right or both, forepaws. This task is easy for the rats to perform, non-stressful, and allows assessment of the rat's ability to use both the injured and non-injured forepaw for weight support and balance. We include data from this task in Figures 5 and 8.

Year 1, Aim 1 - Task 1 Results:

Effects of dAIH on ladder performance in SCI rats

Our findings indicate that AIH treatment alone, without concomitant motor training, does not improve recovery on the ladder task in SCI rats (Figure 1). Rats with transection of the left dorsolateral funiculus were subject to either AIH or normoxia treatment at 4 weeks post-surgery. The data in Figure 1 shows results obtained from methods proposed in Aim 1 of this project, in that animals were treated daily with AIH for 5 days but recording of ladder performance was only carried out on the 5th day of treatment, not on each day of treatment. With this protocol, there was no difference in ladder performance between AIH and normoxia-treated control animals at any timepoint (Figure 1A). In order to determine if severity of injury was important in determining recovery (as we have demonstrated in another paradigm, discussed below), we analyzed the effect of AIH and normoxia on animals with $\geq 50\%$ errors (moderate to severe injury) on the ladder following surgery only. As shown in Figure 1B, there was still no difference in ladder performance between AIH and normoxia treated groups.

Effects of dAIH on grip strength in SCI rats

Our preliminary data and initial experiments focused on assessment of forepaw use in the ladder walking task. We have also assessed forepaw grip strength to investigate whether AIH might promote recovery in more than one task, e.g. to determine whether AIH-induced recovery is robust. We assessed grip strength in SCI rats which received daily AIH treatment for 5 days and compared these to normoxia-treated control animals (Figure 2). There was no difference in grip strength of the left or right forepaw between AIH and normoxia-treated animals at any time point (Figures 2A and 2B).

Year 2, Aim 2 - Task 5 Results:

Effects of dAIH combined with treadmill training on ladder performance in SCI rats

Our findings indicate that AIH treatment in combination with treadmill training does not improve recovery on the ladder task in SCI rats (Figure 3). Rats with transection of the left dorsolateral funiculus were subject to 1 of possible 4 combinations of treatment and motor training at 4 weeks post-surgery. The data in Figure 3 shows results obtained from methods proposed in Aim 2 of this project, in that animals were treated daily with either AIH + treadmill, AIH alone, normoxia + treadmill, or normoxia alone. Recording of ladder performance was only carried out on the last day of treatment (D5), not on each day of treatment. With this protocol, there was no difference in ladder performance between groups at any timepoint (Figure 3A). As discussed below, the extent of injury was shown to have an effect on recovery of AIH treated animals in a specific experimental paradigm. Therefore, we examined the effect of treatment with daily treadmill training on only those animals with $\geq 50\%$ deficit pre-treatment. As shown in Figure 3B, there does not appear to be an effect of treatment with treadmill training on animals with a moderate to severe injury.

Effects of dAIH combined with treadmill training on grip strength in SCI rats

Forepaw grip strength was also assessed in order to investigate whether AIH combined with motor training might promote recovery in more than one task. We assessed and compared grip strength in SCI rats which received either AIH + treadmill, AIH alone, normoxia + treadmill, or normoxia alone (Figure 4). With this protocol, there was no difference in grip strength between groups at any timepoint (Figure 4A and B).

Effects of dAIH combined with treadmill training on paw preference in SCI rats

We assessed and compared paw preference, specifically measuring the use of the left forelimb for weight support and balance, in SCI rats which received either AIH + treadmill, AIH alone, normoxia + treadmill, or normoxia alone (Figure 5). With this protocol, there was no difference in left forepaw usage between groups at any timepoint (Figure 5).

Effects of dAIH combined with daily ladder training on ladder performance in SCI rats.

Preliminary findings in our laboratory indicated that AIH in combination with daily ladder training improved performance on the ladder task in SCI rats. We sought to replicate and further characterize this finding by investigating the effect of lesion severity on recovery after AIH treatment. The data in Figure 6 shows results obtained when we repeated the methods used to obtain our preliminary data, namely that ladder performance was recorded for **each** day of the 7 day AIH treatment. In our laboratory, recording of ladder performance constitutes a motor training session as well, because rats repeatedly cross a horizontal ladder 10 - 15 times during digital videotaping for assessment of performance. Figure 6A shows results for rats with $\geq 50\%$ footslip errors at 4 weeks post-surgery (moderate to severe injury), just prior to onset of AIH treatment. We found AIH treatment plus daily ladder training resulted in significantly fewer errors on the ladder compared with animals receiving ladder training and normoxia treatment at several time points post-treatment (Figure 6A). The data in Figure 6B is important because it demonstrates that we can

replicate our preliminary findings. For rats with minor functional deficits at 4 weeks post-surgery, <50% errors on the ladder prior to treatment, AIH had no effect (Figure 6B).

Effects of dAIH combined with daily ladder training on grip strength in SCI rats

Forepaw grip strength was assessed in order to determine whether AIH combined with daily ladder training would promote recovery in this task as well. We assessed and compared grip strength in SCI rats which received either AIH + daily ladder training or normoxia + daily ladder training (Figure 7). With this protocol, there was no difference in grip strength between groups at any timepoint (Figure 7A and B).

Effects of dAIH combined with daily ladder training on paw preference in SCI rats

We assessed and compared paw preference, specifically measuring the use of the left forelimb for weight support and balance, in SCI rats which received either AIH + daily ladder training, or normoxia + daily ladder training. With this protocol, there was no difference in left forepaw usage between groups at any timepoint (Figure 8A). We also examined only those animals with $\leq 30\%$ left wall contacts at 4wk post-surgery, prior to treatment. There was no difference in left forepaw usage between groups at any timepoint (Figure 8B).

KEY RESEARCH ACCOMPLISHMENTS:

- Our experiments indicate that acute intermittent hypoxia (AIH) elicits sustained improvement in skilled limb use during a ladder walking task when combined with daily ladder training in rats with a moderate to severe SCI. The lack of sustained recovery seen when AIH was administered without concomitant motor training, or when AIH was combined with daily treadmill training suggests that AIH promotes recovery only when combined with task-specific training in a rat model of incomplete cervical SCI.
- Our experiments indicate that AIH does not facilitate recovery of forepaw grip strength or spontaneous forepaw use in a rat model of incomplete cervical SCI.

REPORTABLE OUTCOMES:

We have applied for and successfully received a research grant from the Saskatchewan Health Research Foundation (SHRF Spinal Cord Injury Research) based on our findings from this project. Specifically, we have proposed to investigate the minimum number of days of AIH treatment sufficient to produce sustained functional recovery in a rat model of SCI.

CONCLUSION

In summary, our results demonstrate that AIH, in combination with daily motor training, elicits sustained improvement in skilled limb use during the ladder walking task in a rat model of SCI. Spinal-injured rats with a moderate to severe functional deficit, which underwent AIH treatment and daily motor training, in the form of repeated horizontal ladder crossings, made fewer footslip errors on the ladder for up to 4 weeks after the end of treatment when compared to normoxia-treated, motor-trained control rats. Rats with a less severe functional deficit (<50% errors on the ladder walking task prior to treatment) do not appear to benefit from AIH treatment. As well, spinal-injured rats treated with AIH without concomitant motor training or with daily treadmill training did not show recovery on the ladder task compared to normoxia-treated SCI control rats, regardless of severity of injury. This suggests that task-specific training is required for AIH to elicit recovery of motor function in our rat model of SCI. Nevertheless, it is unclear whether the lack of functional benefit derived from combined dAIH and daily treadmill training is the result of non-task specificity of the exercise, or the stress induced by treadmill running in this group of rats. In the future, we plan to examine this question using voluntary wheel running as a non-stressful form of exercise.

In summary, we have further characterized the contribution of AIH treatment to functional recovery in a rat model of cervical SCI. Our positive findings are important because they reveal that we can obtain consistent effects in an animal model for a promising SCI therapy that is also feasible, in that AIH has already been shown to augment motor function in persons with SCI⁴. Thus, we have a translational framework with which to continue to investigate the effects of AIH on voluntary limb function following chronic spinal cord injury.

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APPENDICES

None

SUPPORTING DATA

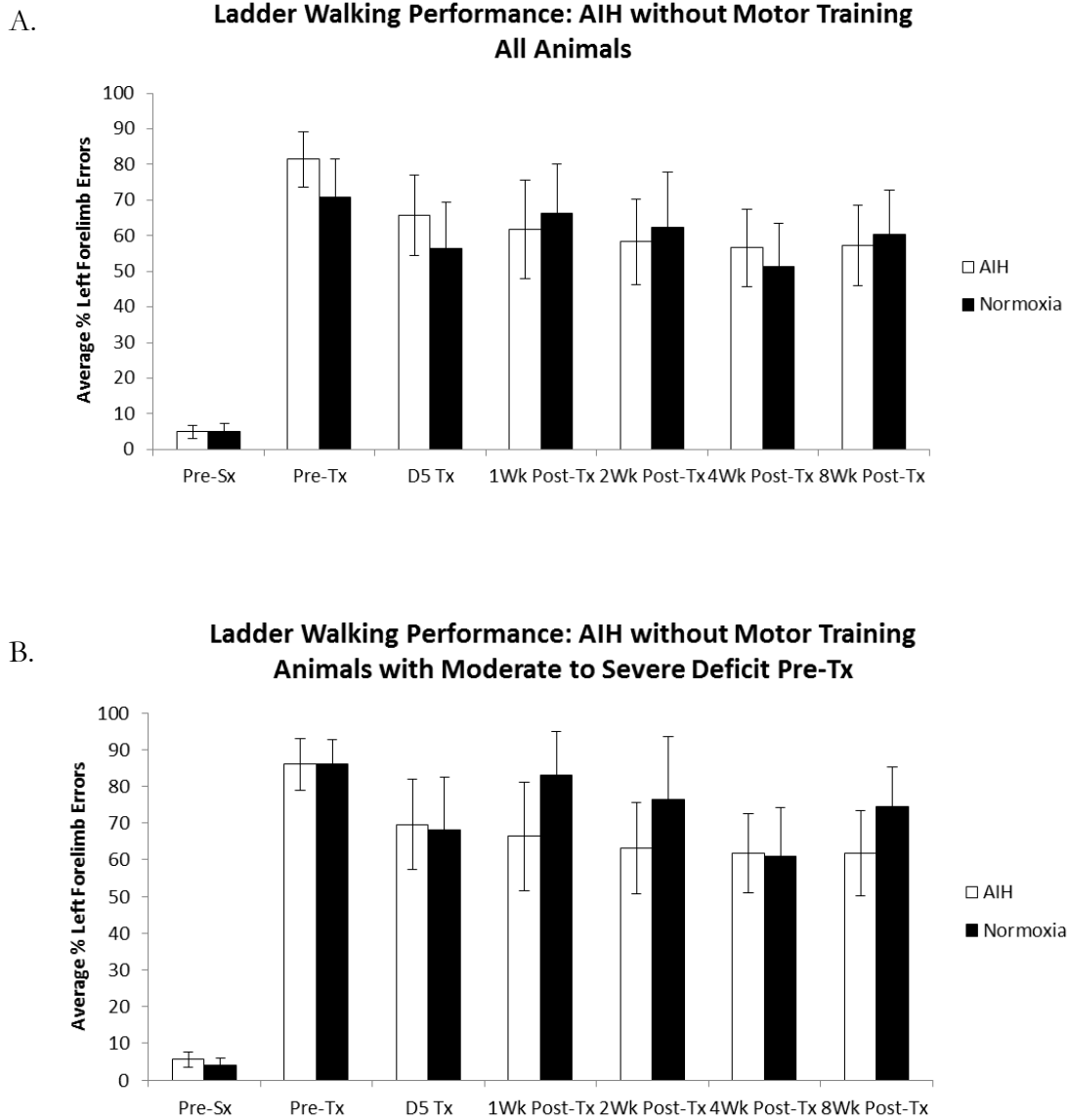
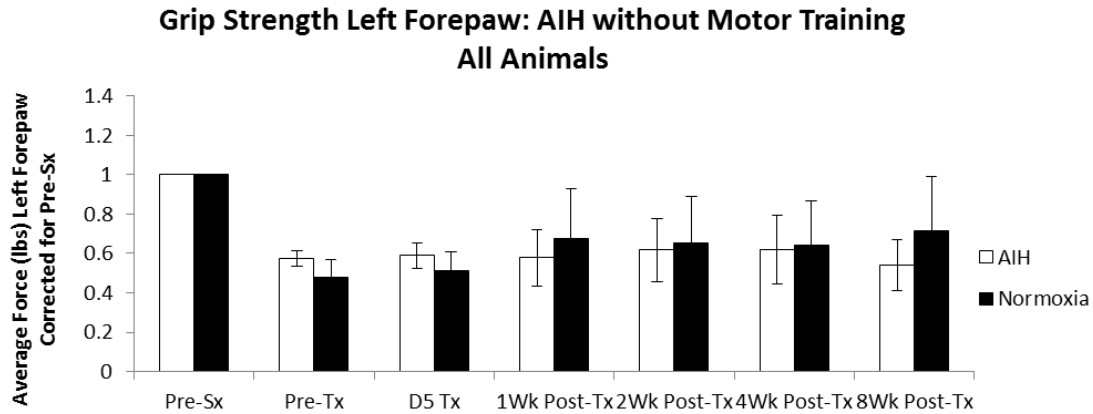


Figure 1: Ladder walking performance in SCI rats exposed to AIH without motor training.

A. All rats which received AIH but not motor training during the week of treatment, regardless of deficit, showed no difference in left forelimb errors during ladder walking when compared to normoxia controls (RM-ANOVA, $p > 0.05$; $n=9$ AIH, $n=7$ normoxia). **B.** Rats which had $\geq 50\%$ errors on the ladder walking task (moderate to severe deficit) prior to treatment and received AIH but not motor training during the week of treatment showed no difference in left forelimb errors during ladder walking when compared to normoxia controls (RM-ANOVA, $p > 0.05$; $n=8$ AIH, $n=5$ normoxia). Treatments began 4wk after cervical spinal cord injury and consisted of daily AIH exposure (5min 11% oxygen alternating with 5min 20% oxygen, room air, repeated 10 times), for 5 days, while normoxia-treated control animals were exposed to 20% oxygen continuously for the same duration. Ladder walking performance was assessed before surgery, pre-treatment (4wk after surgery), on the last day of treatment (D5), and at 1, 2, 4, and 8wk following treatment. Percent forelimb errors = # footslips/total number of steps. Sx = left dorsolateral spinal funiculus transection at C2, Tx = treatment (AIH or normoxia), D = day of treatment. Only effects of treatment are presented here, not effects of time.

A.



B.

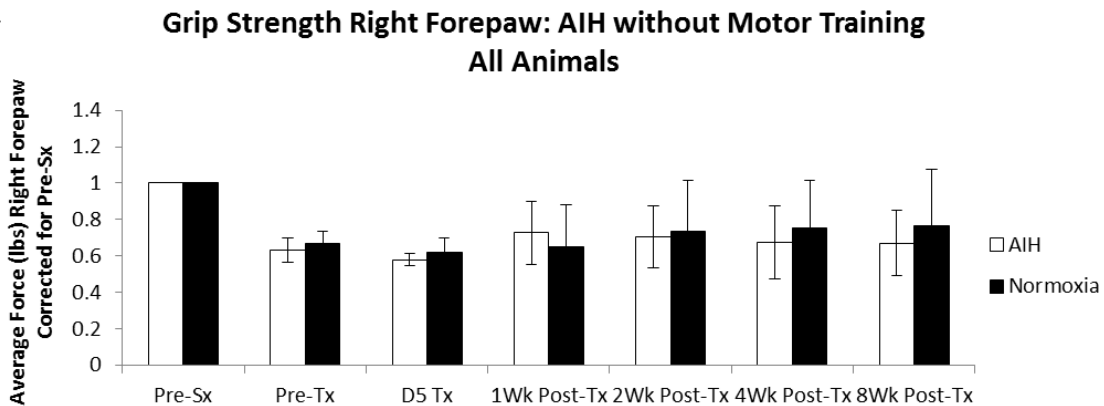


Figure 2: Grip strength performance of left (A) and right (B) forepaws in SCI rats exposed to AIH. All rats which received AIH but no motor training during the week of treatment, regardless of deficit, showed no difference in grip strength of the left (A) and right (B) forepaw when compared to normoxia controls (RM-ANOVA, $p > 0.05$; $n=10$ AIH, $n=8$ normoxia). Treatments began 4wk after cervical spinal cord injury, and consisted of daily AIH exposure (5min 11% oxygen alternating with 5min 20% oxygen, room air, repeated 10 times), for 5 days, while normoxia-treated control animals were exposed to 20% oxygen continuously for the same duration. Grip strength performance was assessed before surgery, before treatment (4wk after surgery), on the last day of treatment (D5), and at 1, 2, 4, and 8wk following treatment. Sx = left dorsolateral spinal funiculus transection at C2, Tx = treatment (AIH or normoxia), D = day of treatment. Only effects of treatment are presented here, not effects of time.

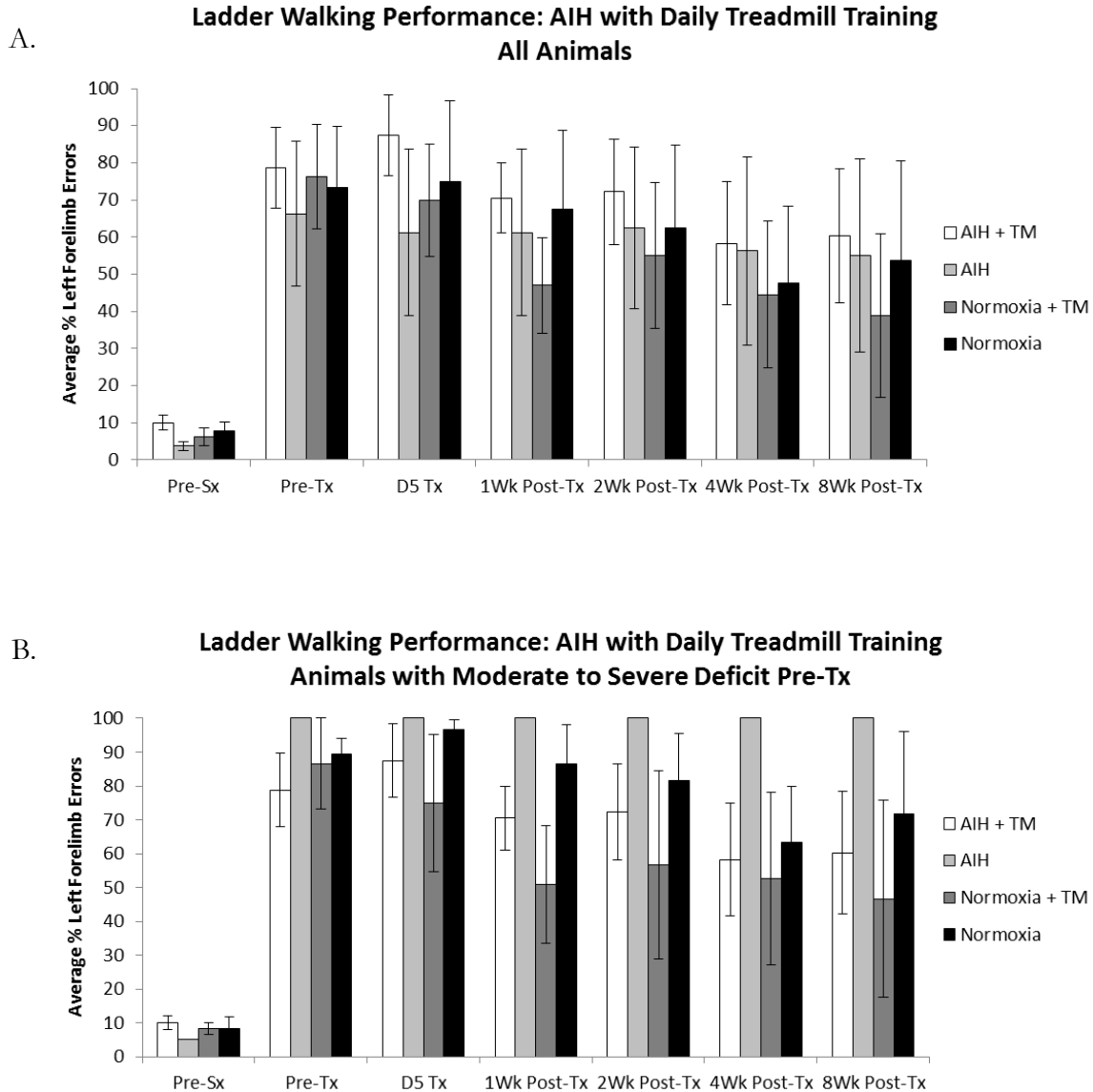
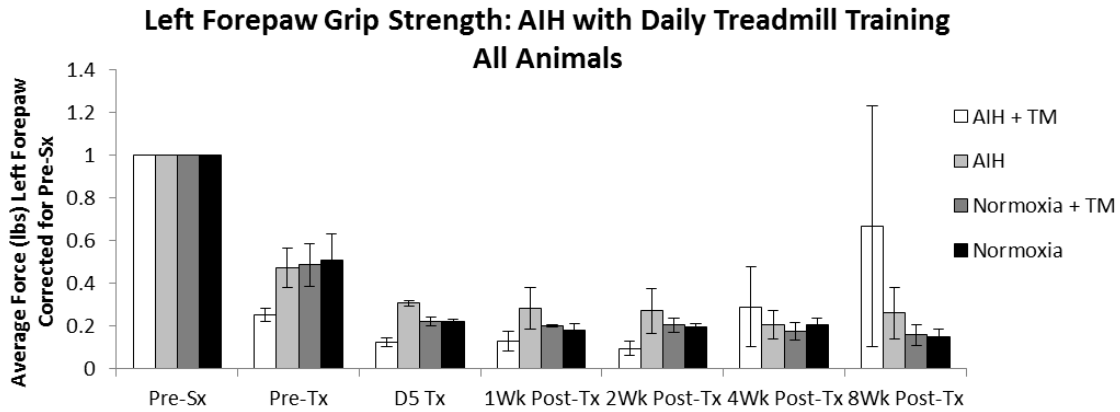


Figure 3: Ladder walking performance in SCI rats exposed to AIH plus treadmill training.

A. All rats which received AIH plus treadmill motor training during the week of treatment, regardless of deficit, showed no difference in left forelimb errors during ladder walking when compared to normoxia controls (RM-ANOVA, $p > 0.05$; $n=4$ AIH + TM, $n=4$ AIH, $n=4$ normoxia + TM, $n=4$ normoxia). **B.** Rats which had $\geq 50\%$ errors on the ladder walking task (moderate to severe deficit) prior to treatment and received AIH plus treadmill motor training during the week of treatment showed no difference in left forelimb errors during ladder walking when compared to normoxia controls (RM-ANOVA, $p > 0.05$; $n=4$ AIH + TM, $n=2$ AIH, $n=3$ normoxia + TM, $n=3$ normoxia). Treatments began 4wk after cervical spinal cord injury and consisted of daily AIH exposure (5min 11% oxygen alternating with 5min 20% oxygen, room air, repeated 10 times), for 5 days, while normoxia-treated control animals were exposed to 20% oxygen continuously for the same duration. On each day of treatment, rats ran on the treadmill 2hr before and again 2hr after treatment, for 20min /session. Ladder walking performance was assessed before surgery, pre-treatment (4wk after surgery), on the last day of treatment (D5), and at 1, 2, 4, and 8wk following treatment. Percent forelimb errors = # footslips/total number of steps. Sx = left dorsolateral spinal funiculus transection at C2, Tx = treatment (AIH or normoxia), D = day of treatment, TM = treadmill. Only effects of treatment are presented here, not effects of time.

A.



B.

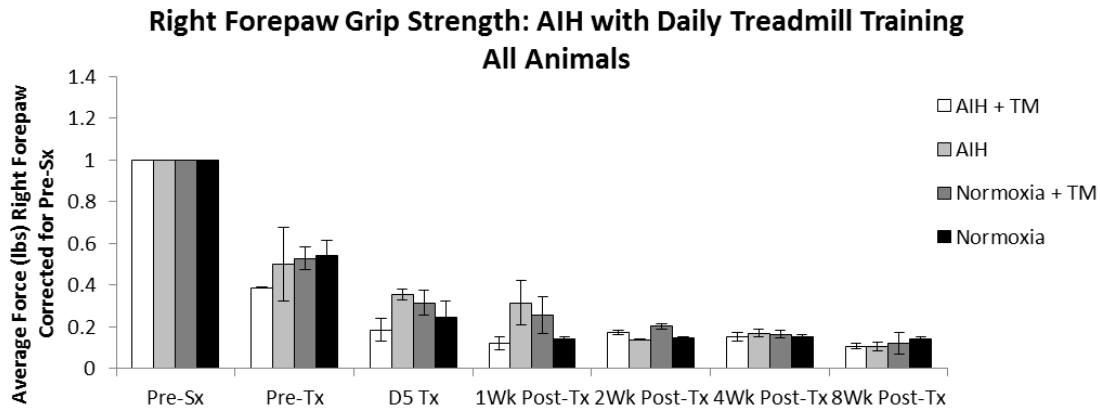


Figure 4: Grip strength performance of left (A) and right (B) forepaws in SCI rats exposed to AIH plus treadmill training. All rats which received AIH plus treadmill motor training during the week of treatment, regardless of deficit, showed no difference in grip strength of the left (A) and right (B) forepaw when compared to normoxia controls (statistical analysis was not performed due to small sample size; n=2 AIH + TM, n=2 AIH, n=2 normoxia + TM, n=2 normoxia). Treatments began 4wk after cervical spinal cord injury, and consisted of daily AIH exposure (5min 11% oxygen alternating with 5min 20% oxygen, room air, repeated 10 times), for 5 days, while normoxia-treated control animals were exposed to 20% oxygen continuously for the same duration. On each day of treatment, rats ran on the treadmill 2hr before and again 2hr after treatment, for 20min/session. Grip strength performance was assessed before surgery, before treatment (4wk after surgery), on the last day of treatment (D5), and at 1, 2, 4, and 8wk following treatment. Sx = left dorsolateral spinal funiculus transection at C2, Tx = treatment (AIH or normoxia), D = day of treatment, TM = treadmill. Only effects of treatment are presented here, not effects of time.

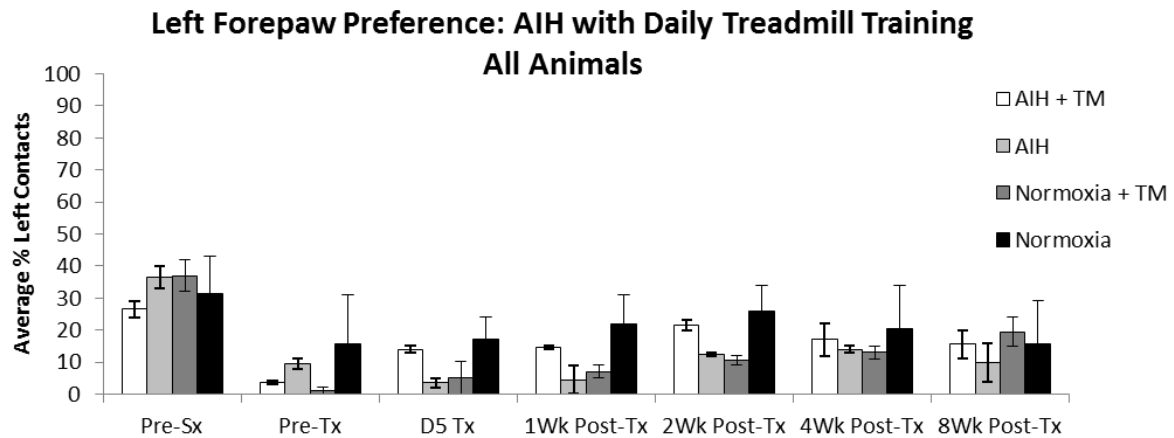


Figure 5: Left forepaw performance in SCI rats exposed to AIH plus treadmill training. All rats which received AIH plus treadmill motor training during the week of treatment, regardless of deficit, showed no difference in use of the left forepaw when compared to normoxia controls (statistical analysis was not performed due to small sample size; $n=2$ AIH + TM, $n=2$ AIH, $n=2$ normoxia + TM, $n=2$ normoxia). Treatments began 4wk after cervical spinal cord injury, and consisted of daily AIH exposure (5min 11% oxygen alternating with 5min 20% oxygen, room air, repeated 10 times), for 5 days, while normoxia-treated control animals were exposed to 20% oxygen continuously for the same duration. On each day of treatment, rats ran on the treadmill 2hr before and again 2hr after treatment, for 20min/session. Paw performance was assessed before surgery, before treatment (4wk after surgery), on the last day of treatment (D5), and at 1, 2, 4, and 8wk following treatment. Sx = left dorsolateral spinal funiculus transection at C2, Tx = treatment (AIH or normoxia), D = day of treatment, TM = treadmill. Only effects of treatment are presented here, not effects of time.

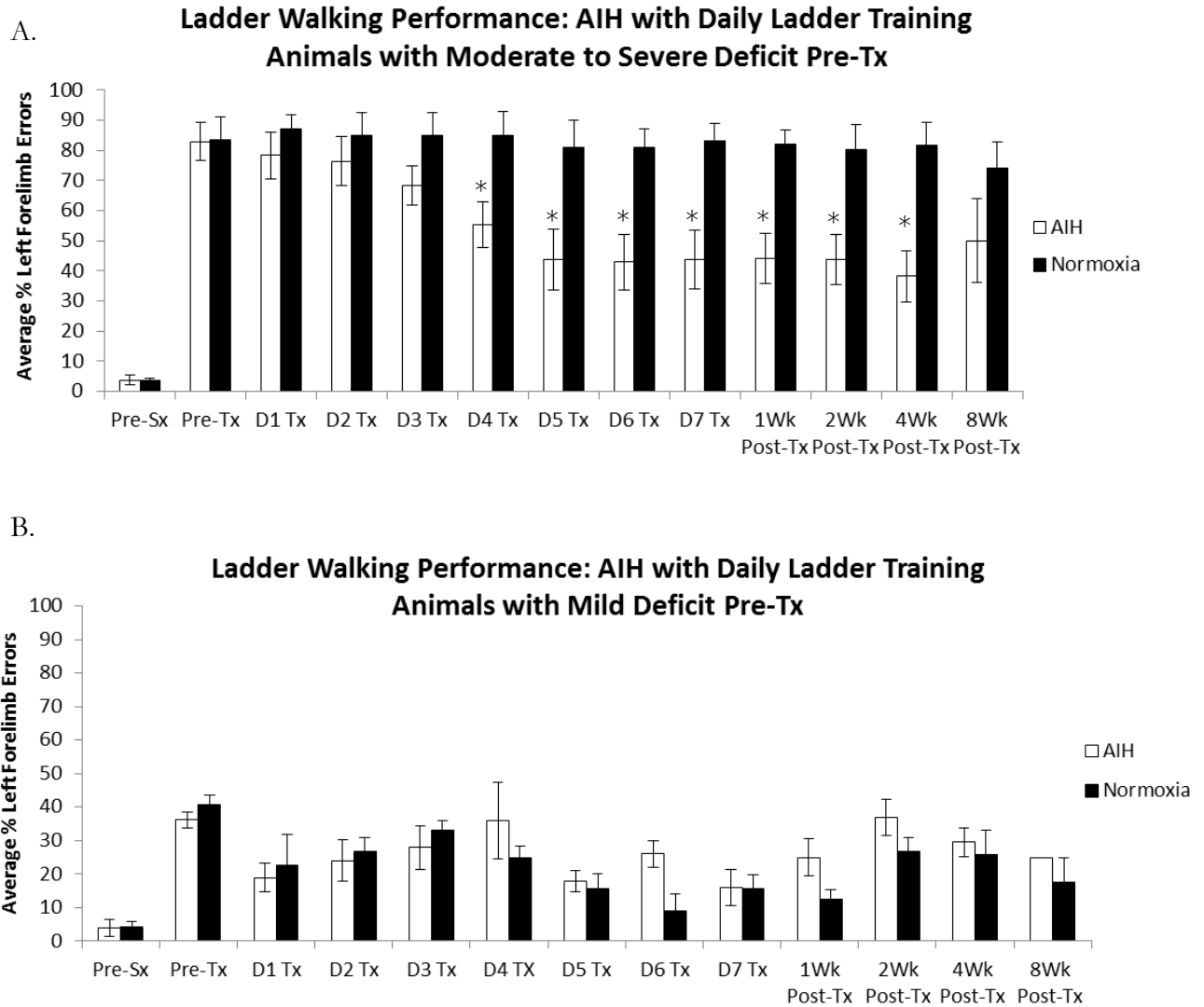


Figure 6: Ladder walking performance in SCI rats exposed to AIH plus ladder training. **A.** Rats which had $\geq 50\%$ errors on the ladder walking task (moderate to severe deficit) prior to treatment and received AIH plus motor training in the form of ladder walking during the week of treatment made fewer left forelimb errors when compared to animals which received the same training and normoxia treatment (*AIH group had significantly fewer errors on the ladder than normoxia group. ANOVA $p < 0.05$; $n=12$ AIH, $n=10$ normoxia with the exception of 8wk post-tx time point where $n=5$ AIH, $n=6$ normoxia). **B.** Rats which had $< 50\%$ errors on the ladder walking task (mild deficit) prior to treatment and received AIH plus motor training in the form of ladder walking during the week of treatment showed no difference in left forelimb errors during ladder walking when compared to normoxia controls (RM-ANOVA, $p > 0.05$; $n=5$ AIH, $n=6$ normoxia with the exception of 8wk post-tx time point where $n=2$ AIH, $n=2$ normoxia). Treatments began 4wk after cervical spinal cord injury and consisted of daily AIH exposure (5min 11% oxygen alternating with 5min 20% oxygen, room air, repeated 10 times), for 5 days, while normoxia-treated control animals were exposed to 20% oxygen continuously for the same duration. Ladder walking performance was assessed before surgery, pre-treatment (4wk after surgery), on each day of treatment, and at 1, 2, 4, and 8wk following treatment. Percent forelimb errors = # footslips/total number of steps. Sx = left dorsolateral spinal funiculus transection at C2, Tx = treatment (AIH or normoxia), D = day of treatment. Only effects of treatment are presented here, not effects of time.

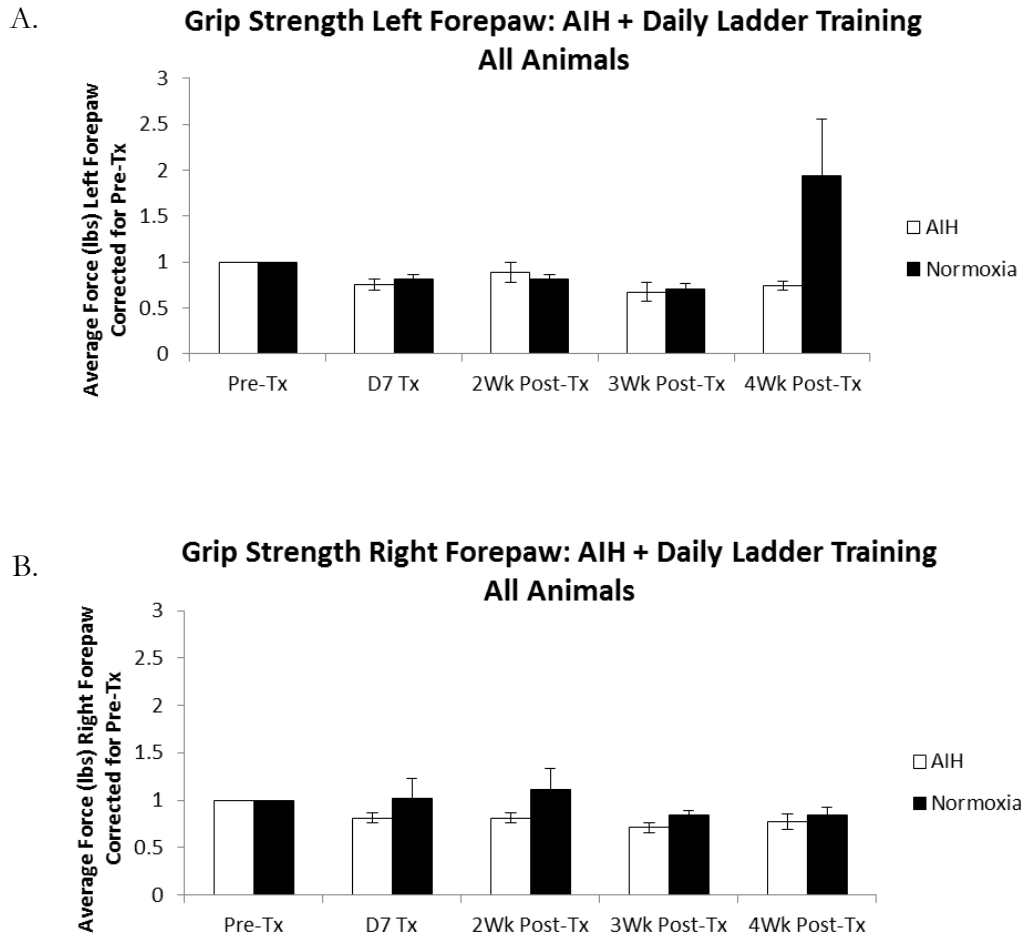
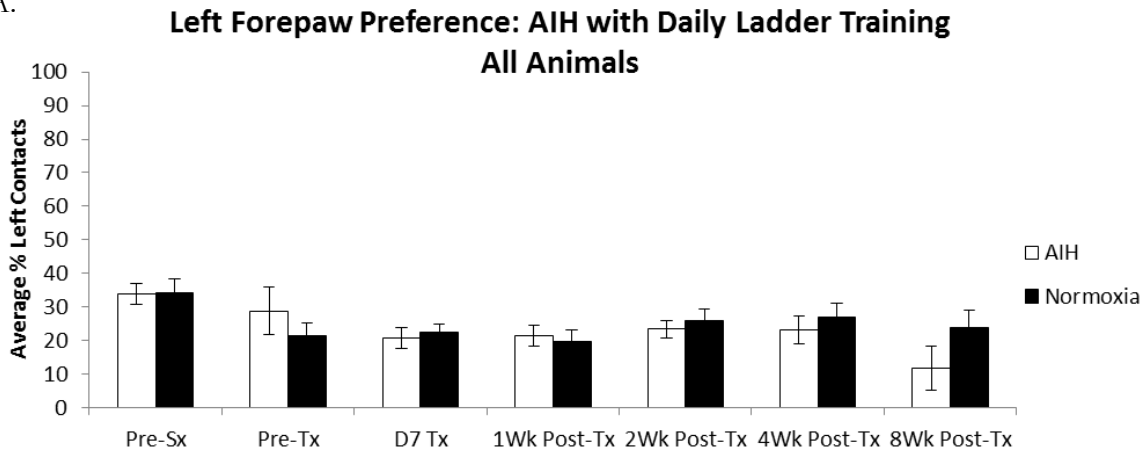


Figure 7: Grip strength performance of left (A) and right (B) forepaws in SCI rats exposed to AIH plus ladder training. All rats which received AIH plus motor training in the form of ladder walking during the week of treatment, regardless of deficit, showed no difference in grip strength of the left (A) and the right (B) forepaw when compared to normoxia controls (RM-ANOVA, $p > 0.05$; $n=3$ AIH, $n=3$ normoxia). Treatments began 4wk after cervical spinal cord injury, and consisted of daily AIH exposure (5min 11% oxygen alternating with 5min 20% oxygen, room air, repeated 10 times), for 7 days, while normoxia-treated control animals were exposed to 20% oxygen continuously for the same duration. Grip strength performance was assessed before treatment (4wk after surgery), on the last day of treatment (D7), and at 2, 3, and 4wk following treatment. Sx = left dorsolateral spinal funiculus transection at C2, Tx = treatment (AIH or normoxia), D = day of treatment. Only effects of treatment are presented here, not effects of time. **NOTE:** Differences as compared to previous grip strength data graphs: larger scale on y-axis, data is corrected for pre-tx time point as pre-sx time point is not available, and there is no 1wk post-tx time point and is a 3wk post-tx time point.

A.



B.

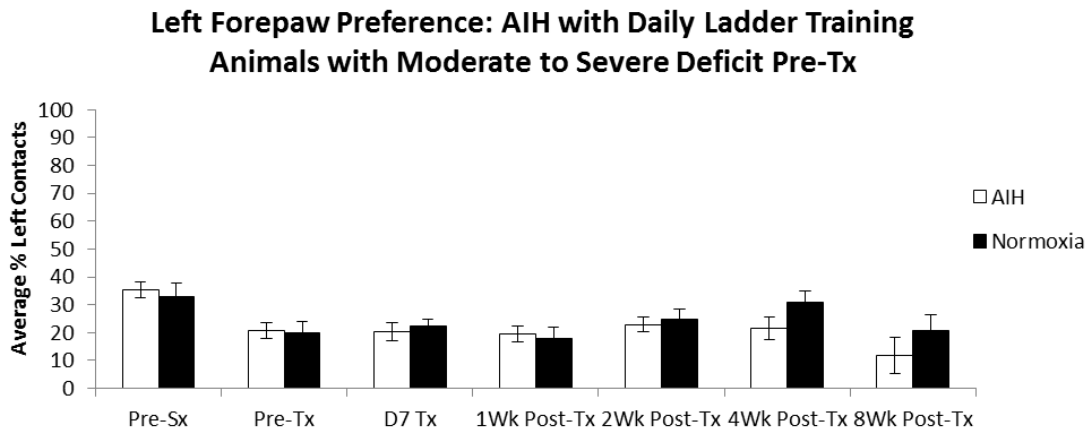


Figure 8: Paw preference performance of the left forepaw in SCI rats exposed to AIH plus ladder training. **A.** All rats which received AIH plus motor training in the form of ladder walking during the week of treatment, regardless of deficit, showed no difference in the use of the left forepaw when compared to normoxia controls (RM-ANOVA, $p > 0.05$; $n=12$ AIH, $n=10$ normoxia with the exception of the 8wk post-tx time point where $n=3$ AIH, $n=4$ normoxia). **B.** Rats which had $\leq 30\%$ left wall contacts (moderate to severe deficit) prior to treatment and received AIH plus motor training in the form of ladder walking during the week of treatment showed no difference in the use of the left forepaw when compared to normoxia controls (RM-ANOVA, $p > 0.05$; $n=10$ AIH, $n=7$ normoxia with the exception of the 8wk post-tx time point where $n=3$ AIH, $n=3$ normoxia). Treatments began 4wk after cervical spinal cord injury, and consisted of daily AIH exposure (5min 11% oxygen alternating with 5min 20% oxygen, room air, repeated 10 times), for 7 days, while normoxia-treated control animals were exposed to 20% oxygen continuously for the same duration. Paw performance was assessed before treatment (4wk after surgery), on the last day of treatment (D7), and at 2, 3, and 4wk following treatment. Sx = left dorsolateral spinal funiculus transection at C2, Tx = treatment (AIH or normoxia), D = day of treatment. Only effects of treatment are presented here, not effects of time.